Non-Peptide Ligands for Opioid Receptors. Design of κ-Specific Agonists

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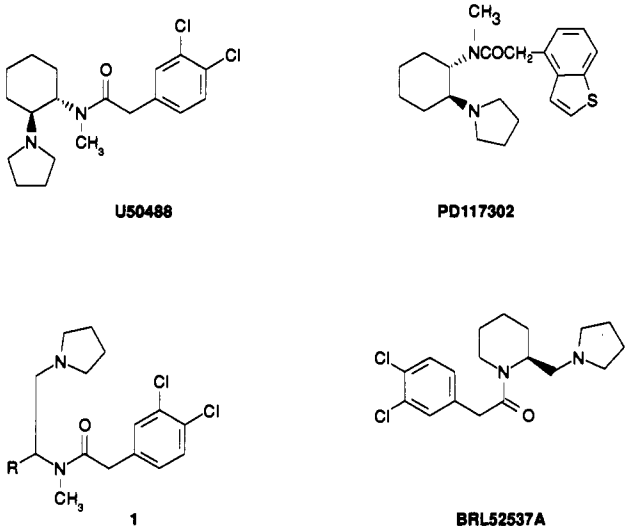
A series of phenyl carboxy esters **5a–d** derived from *N*-(cyclopropylmethyl)normetazocine was synthesized and evaluated for its selectivity at μ, κ, and δ opioid receptors. Compound **5a**, although 43 times less potent than the reference compound U50488, was specific for κ receptors, having no detectable affinity for either μ or δ receptors. Greater binding affinity was seen with the diastereoisomer having the 1′*R*,2′*S* stereochemistry in the cyclopropyl ring of the nitrogen substituent, which was only 12 times less active than U50488. Antinociceptive activity in the mouse tail flick was only slightly lower than that of U50488 (ED_{50} = 7.66 *vs* 4.52 mg/kg). Naloxone fully prevented antinociception induced by (1′*R*,2′*S*)-**5a** at the doses of 2.0 mg/kg. Compound (1′*R*,2′*S*)-**5a** is one of the most κ-selective non-peptide compounds reported to date. The implications of these results in terms of requirements for κ ligands are discussed.

Despite their common N-terminal tetrapeptide fragment, endogenous opioid peptides activate different receptor subpopulations.[1-3] It has been proposed that such a sequence carries a "message" responsible for mediating the opioid effect, while the different C-terminal segments play an "address" role in conferring preference for different receptor subtypes.[4] In effect, the Phe[4] residue, initially included in the "message" sequence,[5] has been relegated to the "address" segment,[6] with the consequence that, in principle, morphine-like pharmacophores, mimicking the Tyr[1] residue of endogenous ligands, might support suitable fragments able to modulate receptor selectivity.

As several studies have established that with the activation of κ opioid receptors it is possible to elicit analgesia without some of the undesired opioid effects of morphine,[7-9] the above strategy has been applied by several researchers in order to obtain κ selective compounds. Nevertheless, although relevant antagonists, such as nor-BNI,[10] have been obtained, only one hybrid peptide–alkaloid[11] derived from β-naltrexamine emerged as a significant κ agonist. The most selective compounds available to date come from studies made on compounds which seem to be structurally unrelated with morphine congeners (e.g. *N*-(aminocyclohexyl)arylacetamides, such as U50488[12] and PD117302,[13] *N*-[2-(1-pyrrolinidinyl)-1-substituted-ethyl]acetamides[14] 1 and 1-(arylacetyl)-2-(aminomethyl)piperidines,[15] such as BRL52537A, Chart I).

An explanation for the relatively unsuccessful applications of the message–address concept in obtaining κ-selective agonists could lie in the fact that the N-terminal tetrapeptide fragment, although structurally identical in all the mammalian opioid endogenous ligands, might assume different conformations in interacting with different receptor subpopulations. In this case, a key role for selectivity should be played by the relative conformations of the functional groups present in the Tyr[1] and the Phe[4] residues.[16] The C-terminal fragment could be important in imposing a particular conformation on the N-terminal fragment by eventually interacting in a specific way with the cell surface in the vicinity of receptors and thus assisting the tetrapeptide fragment in its selective presentation to κ-specific binding sites.[17]

Chart I



In an effort to further explore these hypotheses and with the aim of developing a selective non-peptide ligand for κ-opioid receptors, we designed and synthesized a series of phenyl carboxy esters **5a–d** derived from *N*-(cyclopropylmethyl)normetazocine.[18]

These novel compounds were evaluated in opioid *in vitro* μ/κ/δ receptor binding and in *in vivo* tail flick assays.

Design Rationale

The design strategy of our compounds involved the following considerations. Conformational studies performed in solution on κ-selective dynorphin(1–13) analogues[19] indicate the prevalence, at least for the first four residues, of a nearly fully extended conformation with a consequent intramolecular distance between the phenolic ring of Tyr[1] and the phenyl ring of Phe[4] of about 15 Å. Nevertheless, several authors tend to support the hypothesis that such ligands increase the helix content in their structure on contact with biological membranes.[17] On the other hand, conformational studies on reported

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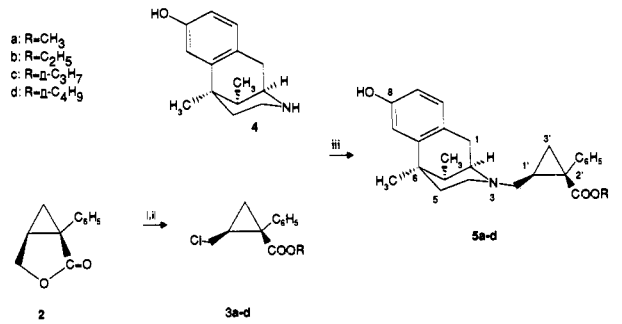
Scheme I[a]



[a] Reagents and conditions: (i) $SOCl_2$, C_6H_6, reflux; (ii) ROH/HCl, room temperature, 12 h, 3 N HCl; (iii) ROH, 80 °C, 36 h, 30 psi.

κ-selective ligands,[15] such as BRL52537A and related U50488, PD117302, and compound 1, which of course do not contain a phenolic residue, permit derivation by molecular modeling analysis[20] of an approximate distance between the basic nitrogen and the phenyl ring of about 6–8 Å. Such distance parameters do not differ significatively from those reported for ligands binding to μ and δ receptors.[21-23] However, as observed by several authors,[24,25] it is possible that conformational requirements for the phenyl ring mimicking the Phe[4] residue relative to the phenolic nucleus and the basic nitrogen, rather than linear distance parameters, might play a critical role for selectivity.

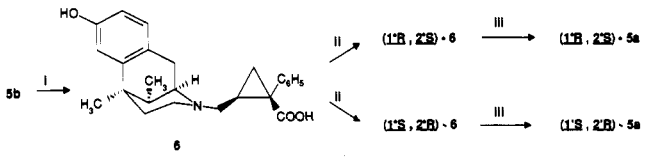
Moreover, recent structure–activity relationships studies on the above cited *N*-(aminocyclohexyl)arylacetamides[26] showed the importance for activity of an amide linkage with stringent conformational requirements with respect to the aromatic moiety.[15] This requirement might implicate the possible involvement of a hydrogen-bonding interaction with an accessory site on the receptor in the proximity of the phenyl ring binding site.

Consequently, we decided to use the (−)-normetazocine[27] nucleus as pharmacophore given its presence in several κ agonists[28] and the fixed and suitable conformation between the phenolic ring and the basic nitrogen. Obviously, a large amount of structure–activity relationship data suggests that the benzomorphan nucleus is also a precursor for μ agonists. Nevertheless, as most of the reported biological tests were of the type which is not addressed to detect receptor selectivity, we resynthesized *N*-(phenylpropyl)normetazocine[29] (PPMT) and used it as a standard in our experiments. The cyclopropylmethyl group, bearing in the 2′ position a phenyl ring and a carboxy ester functionality, was selected as an N-substituent, as it gives linear distance between the basic nitrogen and the phenyl ring close to that suggested above. The carboxy ester group was introduced with the hope to gain information on the putative hydrogen-bonding interaction with an accessory site on the receptor and its stereochemical requirements, as several structure–activity studies on κ-selective agonists derived from benzomorphan indicate a possible hydrogen-bonding interaction of oxygenated N-substituents.[30] The cyclopropylmethyl group also might permit both the phenyl ring and the ester group to assume a relative conformation close to that assumed by the corresponding elements in U50488 analogues.

Chemistry

The synthesis of the esters **5a–d** is outlined in Scheme I. Alkylation of (−)-normetazocine (**4**) with the appropriate *cis*-chloromethyl esters **3a–d**, obtained from lactone **2** by general procedures,[31] was performed in alcoholic solution with potassium bicarbonate in the presence of potassium iodide and afforded the diastereoisomeric mixtures of **5a–d**.

Scheme II[a]



[a] Reagents and conditions: (i) 1 N NaOH, reflux for 5 h followed by neutralization with aqueous HCl; (ii) resolution of the diastereoisomeric mixture by HPLC; (iii) HOBt, DCC, MeOH, 48 h, 10 °C and then 48 h at room temperature.

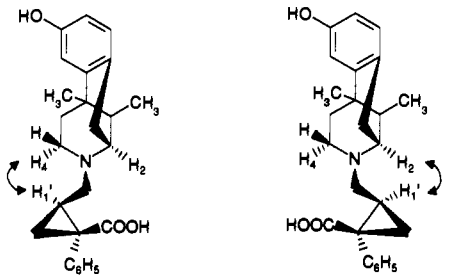
Table I. ^{1}H NMR Data for Compounds (1′*R*,2′*S*)-**6** and (1′*S*,2′*R*)-**6**[a]

	chemical shifts			coupling constants	
	(1′*R*,2′*S*)	(1′*S*,2′*R*)		(1′*R*,2′*S*)	(1′*S*,2′*R*)
H-1ax	2.74	2.75	*J*(1ax,1eq)	18.5	18.5
H-1eq	2.87	2.90	*J*(1ax,2)	6.0	6.0
H-2	3.12	3.28	*J*(1eq,2)	0.0	0.0
H-4ax	2.24	2.20	*J*(2,11)	3.0	3.1
H-4eq	2.91	2.71	*J*(4ax,4eq)	12.5	12.5
H-5ax	1.81	1.79	*J*(4ax,5ax)	12.5	12.5
H-5eq	1.40	1.32	*J*(4ax,5eq)	3.5	3.2
H-7	6.64	6.64	*J*(4eq,5ax)	4.5	4.5
H-9	6.56	6.55	*J*(4eq,5eq)	2.0	2.0
H-10	6.91	6.91	*J*(5ax,5eq)	13.0	12.5
H-11	1.92	1.93	*J*(7,9)	2.5	2.5
H-C_{exo}	3.21	3.28	*J*(9,10)	8.3	8.3
H′-C_{exo}	2.76	2.65	*J*(H-C_{exo},H′-C_{exo})	13.0	13.5
H-1′	1.50	1.45	*J*(H-C_{exo},H-1′)	6.0	5.5
H-3′	1.36	~1.35	*J*(H′-C_{exo},H-1′)	8.0	9.0
H′-3′	1.34	~1.35	*J*(H-1′,H-3′)	*b*	*b*
CH_3-6	1.30	1.30	*J*(H-1′,H′-3′)	*b*	*b*
CH_3-11	0.79	0.79	*J*(H-3′,H′-3′)	*b*	*b*
			J(H-11,CH_3)	7.0	7.0

[a] Chemical shifts are in ppm; coupling constant in Hz; solvent DMSO-d_6. [b] Not reported: second-order spin system.

The hydrolysis of ethyl ester **5b** afforded the free acid **6**. The diastereoisomers of **6** were separated by preparative HPLC to yield the 1′*R*,2′*S* and 1′*S*,2′*R* diastereoisomers of **6**. As a result of the difficulty found in the separation of the diastereoisomeric mixture of **5a**, (1′*R*,2′*S*)-**5a** and (1′*S*,2′*R*)-**5a** were obtained by esterification of (1′*R*,2′*S*)-**6** and (1′*S*,2′*R*)-**6**, respectively, with DCC and HOBt in methanol (Scheme II). Since no suitable crystals of pure diastereoisomers were obtained for X-ray analysis, tentative configurational assignments of the diastereoisomeric structures were made on the basis of NOE NMR data and molecular mechanics calculations performed on the resolved pair of diastereoisomers (1′*R*,2′*S*)-**6** and (1′*S*,2′*R*)-**6**. For this analysis, the diastereoisomers (1′*R*,2′*S*)-**6** and (1′*S*,2′*R*)-**6** were preferred over the corresponding methyl esters (1′*R*,2′*S*)-**5a** and (1′*S*,2′*R*)-**5a** on the basis that a hydrogen bond between the carboxylic acid group and the piperidine nitrogen would likely form. The interaction is expected to reduce the conformational freedom of the molecule, thus increasing the possibility to detect a NOE interaction between pertinent protons of the N-substituent and the piperidine ring.

The proton chemical shifts and vicinal coupling constants are collected in Table I. The proton spectra were fully assigned from the homocorrelated COSY experiment, while the nuclear Overhauser effects were obtained from the bidimensional phase-sensitive NOESY experiment. The NOESY spectra of the two diastereoisomers are very



(1'R, 2'S) - 6 (1'S, 2'R) - 6

Figure 1. NOE effects observed for compounds (1'*R*,2'*S*)-**6** and (1'*S*,2'*R*)-**6**.

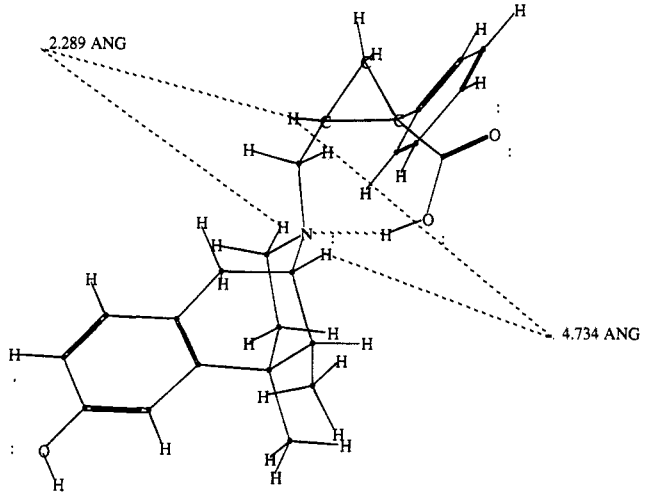


Figure 2. The minimum-energy conformation of (1'*R*,2'*S*)-**6** with the dashed line representing the hydrogen-bonding interaction. Distances between pertinent protons involved in the NOE experiments are also reported.

similar except for one main difference. Compound (1'*R*,2'*S*)-**6** shows a strong NOE between the H-4eq proton of the normetazocine moiety and the H-1' proton of the three-membered ring. For the isomer (1'*S*,2'*R*)-**6** this effect is absent, while a strong NOE contact was detected between the cyclopropane hydrogen H-1' and the H-2 proton of the normetazocine ring (Figure 1). Such NOE data alone did not allow us to distinguish between the two configurational isomers in the absence of basic knowledge of their conformational preferences. Thus, the starting conformation for the geometrical minimization of the two diastereoisomers using the MMX force field was chosen such that the two functionalities in the 2' position almost faced the normetazocine ring system. The rotational conformers around the exocyclic N–CH_2 and CH_2–C1' bonds were carefully examined (10° increments of rotation) for both the axial and the equatorial orientation of the nitrogen substituent. In these computational studies, conformers with a hydrogen-bonding interaction were found to be 5–6 kcal mol^{-1} more stable than any other structure (Figures 2 and 3).

The diastereoisomer with the 1'*R*,2'*S* configuration has an energy minimum conformation with an $r_{H\text{-}1',H\text{-}4eq}$ distance of *ca.* 2.29 Å, fully explaining the strong NOE observed between these two protons for compound (1'*R*,2'*S*)-**6**. On the other hand, the diastereoisomer with the 1'*S*,2'*R* configuration showed an energy minimum with an $r_{H\text{-}1',H\text{-}2eq}$ distance of *ca.* 2.27 Å in agreement with the NOE contact observed for compound (1'*S*,2'*R*)-**6**. While this agrees with experimental and theoretical data, some

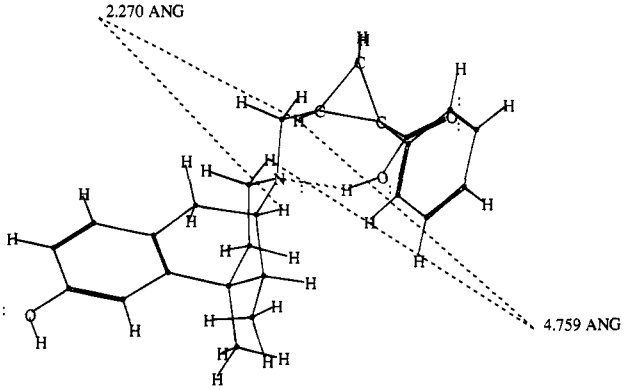


Figure 3. The minimum-energy conformation of (1'*S*,2'*R*)-**6** with the dashed line representing the hydrogen-bonding interaction. Distances between pertinent protons involved in the NOE experiments are also reported.

Table II. Binding Affinity to κ and μ Opioid Receptors

	Opioid Receptor Binding Affinity K_i ± SEM (nM)[a,b]		
compound	κ	μ	μ/κ ratio[c]
5a	866 ± 98	≥25000	*d*
(1'*R*,2'*S*)-**5a**	240 ± 39	≥25000	*d*
(1'*S*,2'*R*)-**5a**	2640 ± 332	≥25000	*d*
5b	4425 ± 282	≥25000	*d*
5c	6428 ± 227	≥25000	*d*
5d	≥25000	≥25000	*d*
6a	≥25000	≥25000	*d*
U50488	20.5 ± 1.4	2128 ± 160	104
EKC	188 ± 35.5	690 ± 55	3.67
PPMT	1116 ± 143.8	825 ± 37.6	0.74
morphine	6424 ± 327	36 ± 12.5 (7)	0.0056

[a] Each K_i value represents the mean from concentration–response curves performed in triplicate (n = 5 experiments) unless otherwise indicated in parentheses. [b] δ opioid receptor affinity for compounds **5a–d** and U50488 is ≥25000 nM (n = 5). The value for morphine is 1220 ± 118 nM (n = 3). [c] μ/κ ratio = $K_i(\mu)/K_i(\kappa)$. [d] Not calculable.

discrepancies exist between the experimental and the computed values of the coupling constants, especially for J(H'-C_{exo},H-1') involving protons in a *trans* relationship. The dihedral angles H-C_{exo}–C_{exo}–C-1'–H-1' and H'-C_{exo}–C_{exo}–C-1'–H-1' derived from these molecular mechanics calculations are for both diastereoisomers *ca.* 49° and 164°, respectively. The corresponding vicinal coupling constants J(H-C_{exo},H-1') and J(H'-C_{exo},H-1') computed through the generalized Karplus equation proposed by Haasnoot *et al.*[32] are *ca.* 5 and 11 Hz, respectively. Experimental values are 6.0 and 8.0 Hz for (1'*R*,2'*S*)-**6** and 5.5 and 9.0 Hz for (1'*S*,2'*R*)-**6**, respectively (see Table I). Of course, these discrepancies can be attributed to the fact that the Karplus equation is not fully applicable in this case, since it is not specifically parameterized for protons belonging to a three-membered ring. Nevertheless, conformations obtained from molecular mechanics calculations were determined *in vacuo* with the amino and carboxylic acid both neutral. The molecules could be more "flexible" in DMSO solution and some minor conformational states (local minima) might cause an averaging of the coupling constants.

Results

Compound **5a**, among the synthesized esters, was found to have the higher κ opioid receptor affinity, but it was 43 times less potent than the prototype κ agonist U50488 (Table II). After separation of the two diastereoisomers, (1'*R*,2'*S*)-**5a** displayed a clearly higher affinity for the κ receptors than (1'*S*,2'*R*)-**5a** (K_i = 240 ± 39 and 2640 ± 332

Table III. Binding Affinity to κ Opioid Receptors in the Absence and the Presence of 120 mM NaCl + 50 μM Gpp(NH)p

compound	K_i ± SEM (nM)[a]	
	-NaCl and Gpp(NH)p	+NaCl and Gpp(NH)p
5a	833 ± 76	2622 ± 166
(1′*R*,2′*S*)-**5a**	224 ± 28	926 ± 34
U50488	16.5 ± 2.3	204 ± 13.7

[a] See Table II.

Table IV. Effect of Naloxone (Nx) on Antinociceptive Activity Evaluated in the Mouse[a]

compound[b]	antinociceptive index[c] (mean ± SEM)
saline + saline[d]	0.06 ± 0.03
5a + saline	1 ± 0[e]
5a + 0.2 mg/kg[d] Nx	0.82 ± 0.10[e]
5a + 2.0 mg/kg Nx	0.16 ± 0.12
U50488 + saline	1 ± 0[e]
U50488 + 0.2 mg/kg Nx	0.78 ± 0.08[e]
U50488 + 2.0 mg/kg Nx	0.15 ± 0.09
morphine + saline	1 ± 0[e]
morphine + 0.2 mg/kg Nx	0.13 ± 0.10
morphine + 2.0 mg/kg Nx	0.09 ± 0.04

[a] Tail flick assay performed in groups of six male Swiss mice (20–25 g). [b] Each compound was administered at 10 mg/kg per 10 mL sc. [c] Antinociceptive index was evaluated 30 min after treatment as follows: time postadministration − time preadministration/10 − time preadministration; cutoff = 10 s. [d] Nx or saline were injected sc 10 min before. [e] $p < 0.01$ *vs* saline-treated group by analysis and Dunnett's test.

nM, respectively) and was only 12 times less potent than U50488 (Table II). U50488 displayed a higher μ/κ selectivity ratio (104) compared to morphine (0.0056), whereas it was not possible to evaluate the μ/κ selectivity ratio for the synthesized compounds, since these did not displace the binding of [^{3}H]diprenorphine from rat brain membranes incubated in the presence of U50488 and DADLE up to the dose of 25 000 nM (Table II). The reference compound PPMT displayed a poor affinity for all receptor subpopulations, and the μ/κ ratio was 0.74 (Table II). K_i values for compounds **5a**, (1′*R*,2′*S*)-**5a**, and U50488 were significantly higher when binding assays were performed in the presence of 120 mM NaCl and 50 μM Gpp(NH)p. In these conditions κ opioid receptors shift into a state with a low affinity for agonists (Table III).

Compound **5a**, U50488, and morphine displayed antinociceptive activity in the mouse tail flick assay. The ED_{50}, evaluated 30 min after sc injection (peak effect), was 7.66 mg/kg (95% CL 4.25–10.52) for compound **5a**, 4.52 mg/kg (95% CL 2.17–7.29) for U50488, and 4.99 mg/kg (95% CL 1.96–6.24) for morphine. Naloxone fully prevented antinociception induced by **5a** and U50488 at the dose of 2.0 mg/kg, but was ineffective at 0.2 mg/kg, whereas morphine antinociception was also antagonized by the lower dose of naloxone (Table IV).

Discussion

Although possessing an affinity for κ receptors which is $^1/_{12}$ that displayed by U50488, (1′*R*,2′*S*)-**5a** displayed a greater selectivity for the κ receptors over the μ and δ sites than any of the standard agents examined.

Comparison of the action profile of compound (1′*R*,2′*S*)-**5a** with those of EKC and PPMT also suggests that even minor structural alterations of the N-substituent are able to modify receptor preferences of benzomorphan derivatives. As several metazocine analogues,[33] such as MR2034 and bremazocine, which contain only an H-bonding group in the nitrogen substituent, show preference for κ receptors but still maintain an important μ component, it seems reasonable that the simultaneous presence of the H-bonding group and the phenyl ring in this particular configuration might be the key element blocking the binding to μ receptors.

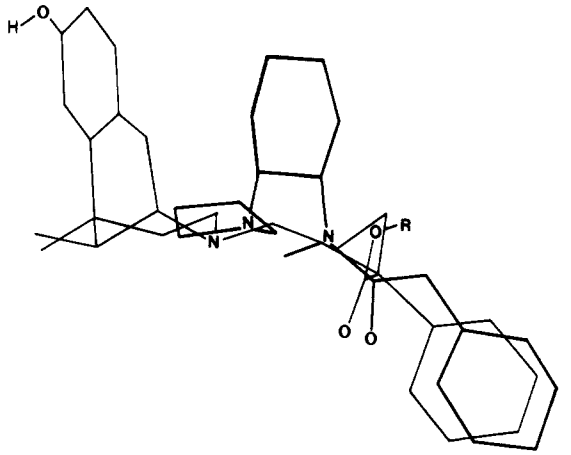


Figure 4. Computer superimposition of one of the stable conformation of (1′*R*,2′*S*)-**5a** (R = CH_3) and that of U50488 showing the carboxy group, the aromatic ring, and the teriary nitrogen matching and overlay lacking between cyclohexane and the lipophilic part of the normetazocine moiety: bold lines, U50488; fine lines, (1′*R*,2′*S*)-**5a**.

The significant difference in the binding potency showed by the two diastereoisomers of **5a** suggests that pronounced stereochemical requirements are necessary for the substituents on the cyclopropane ring. Thus, all the four structural elements (i.e. the basic nitrogen, the two aromatic rings, and the carbomethoxy group) present in (1′*R*,2′*S*)-**5a** seem to have stringent stereochemical preferences. Interestingly, these elements, except of course the phenolic ring, can assume in compound (1′*R*,2′*S*)-**5a** a relative conformation that makes it superimposable on U50488 and its analogues (Figure 4). According to this model, we can suppose that U50488 analogues bind to complementary κ sites where a phenolic ring is not essential for binding. Of course, we do not have at the moment any evidence that compound (1′*R*,2′*S*)-**5a** binds to the same receptor sites as does U50488.

Experimental Section

^{1}H NMR spectra were run on a Bruker AM 500 spectrometer in DMSO-d_6 solution. The NOESY experiments were performed in the phase-sensitive mode with the TPPI method using a mixing time of 400 ms and a repetition delay of 2 s. Molecular modeling calculations were performed with PCMODEL, version 4.0 (Serena Software). Melting points were determined on a Buchi 530 capillary apparatus and are uncorrected. Analytical thin-layer chromatography (TLC) was performed on precoated silica gel 60 F_{254} aluminum sheets (Merck). Analytical HPLC was carried out on a Waters Model 600E. Optical rotations were determined in MeOH solution with a Perkin-Elmer 241 polarimeter. Elemental analyses were measured on an elemental analyzer (Model 1106, Carlo Erba). Molecular weights of the obtained products were determined by MS on a Kratos 2S RFA spectrometer using a Tektronix 4205 computer system. The following abbreviations are used: DCC, *N*,*N*-dicyclohexylcarbodiimide; MS, electron impact mass spectrometry; HOBt, 1-hydroxybenzotriazole; EKC, ethyl ketocyclazocine; HPLC, high-performance liquid chromatography. Physical and analytical data for the compounds **5a–b** and **6** are shown in Table V.

Alkyl (1*R*,2*S*/1*S*,2*R*)-2-(Chloromethyl)-1-phenylcyclopropanecarboxylate (3a–d). To a solution of 1.0 g (5.74 mmol) of lactone of (1*R*,2*S*/1*S*,2*R*)-2-(hydroxymethyl)-1-phenylcyclopro-

Table V. Physical Properties of Phenyl Carboxy Esters 3a–d and 5a–d and acids 6

compd	R	mp, °C	R_f	cryst solv	% yield	formula	$[\alpha]^{25}_D$,[a] deg	MS, m/z (M^+)
3a	CH_3	77–79	0.56[b]		83	$C_{12}H_{13}O_2Cl$[c]		224
3b	C_2H_5	40–42[d]	0.59[b]		72	$C_{13}H_{15}O_2Cl$[c]		238
3c	n-C_3H_7	162–172[e]	0.61[b]		74	$C_{14}H_{17}O_2Cl$[c]		252
3d	n-C_4H_9	152–162[e]	0.64[b]		85	$C_{15}H_{19}O_2Cl$[c]		266
5a	CH_3	169–172	0.52[f]	MeOH/H_2O	48	$C_{28}H_{31}NO_3$[g]		405
(1′R,2′S)-5a	CH_3	181–184	0.62[h]	MeOH/H_2O		$C_{28}H_{31}NO_3$[c]	−131	405
(1′S,2′R)-5a	CH_3	220–221	0.63[h]	MeOH/H_2O		$C_{28}H_{31}NO_3$[c]	+18	405
5b	C_2H_5	152–153	0.57[f]	MeOH/H_2O	52	$C_{27}H_{33}NO_3$[g]		419
5c	n-C_3H_7	185–192	0.58[f]	MeOH/H_2O	43	$C_{28}H_{35}NO_3$[g]		433
5d	n-C_4H_9	177–178	0.60[f]	MeOH/H_2O	49	$C_{29}H_{37}NO_3$[g]		447
6[i]	H	204–209	0.72[j]		89	$C_{25}H_{29}NO_3$[g]		391
(1′R,2′S)-6[k]	H	209–212	0.44[h]			$C_{25}H_{29}NO_3$[c]	−27	391
(1′S,2′R)-6[k]	H	214–215	0.39[h]			$C_{25}H_{29}NO_3$[c]	−6	391

[a] All optical rotations were determined in methanol (c = 1.00). [b] Toluene. [c] Purity evaluated by chromatographic analysis. [d] 44–46 °C in ref 24. [e] Bp 15 mmHg. [f] Toluene–ethyl acetate (2:8). [g] All compounds had satisfactory C, H, and N microanalytical data within ±0.4% of the theoretical value. [h] Chloroform–methanol–acetic acid (8:2:0.05). [i] The diastereoisomeric mixture was used without further purification. [j] n-Butanol–water–acetic acid (4:5:1). [k] Preparative HPLC was performed on a Hypersil ST 5 μm column using chloroform and 2-propanol (70:30) as eluent. Retention times (min) were 12.49 for (1′R,2′S)-6 and 17.56 for (1′S,2′R)-6.

panecarboxylic acid[31] (2) and 16 mg of $ZnCl_2$ in 5 mL of dry benzene was added dropwise at 0 °C under a nitrogen atmosphere 1.3 mL (17 mmol) of thionyl chloride. After refluxing and stirring for 5 h, the reaction mixture was cooled at 0 °C and a solution of 33 mmol of 3 N HCl in solution of the suitable alcohol was added dropwise. The resulting solution was stirred for 12 h at room temperature. The solvent was then evaporated *in vacuo* and the residue dissolved in ethyl ether and washed three times with a 4% solution of $NaHCO_3$. The ether was dried over sodium sulfate and the solvent was evaporated *in vacuo* to leave an oil which was purified by fractional distillation.

(1′*R*,2′*S*/1′*S*,2′*R*)-6,11-Dimethyl-1,2,3,4,5,6-hexahydro-3-[[2′-(alkoxycarbonyl)-2′-phenylcyclopropyl]methyl]-2,6-methano-3-benzazocin-8-ol (5a–d). To a mixture of 300 mg (1.38 mmol) of 6,11-dimethyl-1,2,3,4,5,6-hexahydro-2,6-methano-3-benzazocin-8-ol[21] (4), 174 mg (2.07 mmol) of $NaHCO_3$, and a catalytic amount of KI in 30 mL of the corresponding alcohol were added 1.52 mmol of the suitable alkyl (1*R*,2*S*/1*S*,2*R*)-2-(chloromethyl)-1-phenylcyclopropanecarboxylate (**3a–d**). The reaction mixture was heated in an autoclave at 30 atm at 80 °C for 36 h. The resulting mixture was filtered and the solution evaporated *in vacuo*. The residue was then purified over a silica gel column with chloroform–ethyl acetate as eluent (gradient elution). The fractions containing the desired compound were evaporated to dryness. The residue was crystallized from a mixture of methanol and water.

(1′*R*,2′*S*)-6,11-Dimethyl-1,2,3,4,5,6-hexahydro-3-[(2′-carboxy-2′-phenylcyclopropyl)methyl]-2,6-methano-3-benzazocin-8-ol and (1′*S*,2′*R*)-6,11-Dimethyl-1,2,3,4,5,6-hexahydro-3-[(2′-carboxy-2′-phenylcyclopropyl)methyl]-2,6-methano-3-benzazocin-8-ol (6). To 419 mg (1 mmol) of (1′*R*,2′*S*/1′*S*,2′*R*)-6,11-dimethyl-1,2,3,4,5,6-hexahydro-3-[[2′-(ethoxycarbonyl)-2′-phenylcyclopropyl]methyl]-2,6-methano-3-benzazocin-8-ol (**5b**) was added 3 mL of a solution of 1 N NaOH (3 mmol) and the mixture was refluxed and stirred for 5 h. After neutralization of the solution with dilute HCl, the resulting acid was extracted with chloroform. The chloroformic extracts were dried over sodium sulfate and the solvent was evaporated *in vacuo*. The diastereoisomeric mixture of 6,11-dimethyl-1,2,3,4,5,6-hexahydro-3-[(2′-carboxy-2′-phenylcyclopropyl)methyl]-2,6-methano-3-benzazocin-8-ol (**6**) was resolved on an HPLC Hypersil ST 5-μm column with chloroform and 2-propanol (70:30) as eluent to give the diastereoisomers (1′*R*,2′*S*)-**6** and (1′*S*,2′*R*)-**6**.

(1′*R*,2′*S*)-6,11-Dimethyl-1,2,3,4,5,6-hexahydro-3-[[2′-(methoxycarbonyl)-2′-phenylcyclopropyl]methyl]-2,6-methano-3-benzazocin-8-ol [(1′*R*,2′*S*)-5a] and (1′*S*,2′*R*)-6,11-Dimethyl-1,2,3,4,5,6-hexahydro-3-[[2′-(methoxycarbonyl)-2′-phenylcyclopropyl]methyl]-2,6-methano-3-benzazocin-8-ol [(1′*S*,2′*R*)-5a]. To a solution of 20 mg (0.051 mmol) of (1′*R*,2′*S*)-**6** or (1′*S*,2′*R*)-**6** in 3 mL of MeOH were added 7.6 mg (0.056 mmol) of HOBt and 11.5 mg (0.056 mmol) of DCC. The resulting mixture was stirred for 48 h at 10 °C and for 48 h more at room temperature. Compounds were purified by column chromatography on a silica gel column using CH_2Cl_2–C_6H_6–CH_3OH (85:5:10) as eluent. Final products were crystallized from MeOH–water.

Binding to κ Sites. Crude membrane fraction obtained from male Hartley guinea pig cerebella was used. The membrane fraction suspended in 50 mM Tris-HCl (pH 7.6) at a concentration of 0.1 mg/mL protein was incubated at 25 °C for 60 min with 1 nM [^{3}H]diprenorphine (30 Ci/mmol, Amersham) and various concentrations of compounds.[34,35] Binding studies were performed in the presence of an excess of [D-Ala^2,*N*-MePhe^4,Glyol^5]-enkephalin (DAGO, 100 nM) and [D-Ala^2,D-Leu^5]enkephalin (DADLE, 100 nM) to eliminate interaction with μ and δ receptors, respectively.

Incubation was terminated by the rapid filtration of the mixture through Whatman GF/B filters presoaked in 1% polyethylenimine; the filters were rinsed four times with ice-cold 50 mM Tris-HCl (pH 7.6). The radioactivity retained on the filter was counted by a liquid scintillation counter. Nonspecific binding was determined by the addition of MR2266 (100 nM), a purported κ-receptor antagonist.

In order to evaluate possible influences of Na^+ ions and of guanosine 5′-[β,γ-imido]triphosphate, sodium salt (Gpp(NH)p), on equilibrium saturation binding of the assayed compounds, fixed amounts of 120 mM NaCl and 50 μM Gpp(NH)p were included in a separate set of experiments (see Table III) carried out as described by Frances *et al.*[36]

The inhibition constant (K_i) of each compound was calculated according to the following equation: $K_i = IC_{50}/(1 + [L]/K_d)$, where [L] is the ligand concentration and K_d represents the dissociation constant for [^{3}H]diprenorphine (K_d = 0.55 ± 0.07 nM; n = 5).[37]

Binding to μ and δ Sites. Male albino Sprague–Dawley rats (Charles River, Italia) were sacrificed by decapitation. Brains, minus cerebella, were homogenized in 10 vol of Tris-HCl (pH 7.4) and centrifuged at 40000*g* for 30 min. The final pellets were suspended in 50 mM Tris-HCl (pH 7.6) at a concentration of 0.5 mg/mL protein. To evaluate the specific binding to μ sites, displacement of the binding of [^{3}H]diprenorphine (1 nM) was measured in the presence of U50488 (100 nM) and of DADLE (100 nM) added to saturate the κ and δ opioid receptors, respectively (the K_d of the radioligand was 0.22 ± 0.02 nM; n = 5). Nonspecific binding was determined by the addition of DAGO (100 nM). To evaluate the specific binding to δ sites, the displacement of the binding of [^{3}H]diprenorphine (the K_d of the radioligand was 0.44 ± 0.03 nM; n = 5) was measured in the presence of U50488 (100 nM) and DAGO (100 nM) added to saturate the κ- and μ-opioid receptors, respectively. Binding assays were carried out as previously referred. Nonspecific binding was determined by the addition of DADLE (100 nM).

In Vivo Antinociceptive Activity. The tail flick assay was adopted to evaluate the nociceptive threshold in male Swiss mice (20–25 g). A cutoff time of 8 s was imposed.[38] ED_{50} values and their 25% confidence intervals were determined by using the method of Litchfield and Wilcoxon.[39,40]

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